

Figure 1A

[illegible]

Figure 1B

6	mouse_E3 α II	RYAVDILTWE	KESELPEDLE	VAEKSDTYYC	MLFNDEVHTY	EQVIYTLQKA	249
4	human_E3 α II	RYAVEILTWE	KESELPADLE	MVEKSDTYYC	MLFNDEVHTY	EQVIYTLQKA	249
15	mouse_E3 α I	KYIVEMTIWE	EEKELPPELQ	IREKNERYYC	VLFNDEHHSY	DHVIYSLQRA	248
2	human_E3 α I	KYVVEMTIWE	EEKELPPELQ	IREKNERYYC	VLFNDEHHSY	DHVIYSLQRA	248
	Consensus	.Y.VE...WE	.E.ELP..L.	..EK...YYC	.LFNDE.H.Y	..VI.Y.LQ.A	250
6	mouse_E3 α II	VNCTQKEAIG	FATTVDRDGR	RPVRYGDFQY	CDQAKTVI VR	NTSRQTK-PL	298
4	human_E3 α II	VNCTQKEAIG	FATTVDRDGR	RSVRYGDFQY	CEQAKSVI VR	NTSRQTK-PL	298
15	mouse_E3 α I	LDCELAEAQL	HTTAIDKEGR	RAVKAGVYAT	CQEAKEDI KS	HSENVSQHPL	298
2	human_E3 α I	LDCELAEAQL	HTTAIDKEGR	RAVKAGAYAA	CQEAKEDI KS	HSENVSQHPL	298
	Consensus	..C...EA..	..T..D..GR	R.V..G....	C..AK..I..PL	300
6	mouse_E3 α II	KVQVMHSSVA	AHQNFGLKAL	SWLGSVI GYS	DGLRRI LCQV	GLQEGPDGEN	348
4	human_E3 α II	KVQVMHSSIV	AHQNFGLKLL	SWLGSII GYS	DGLRRI LCQV	GLQEGPDGEN	348
15	mouse_E3 α I	HVEVLHSVVM	AHQKFALRLG	SWWNKI MSYS	SDFRQI FCQA	CLVEEPPGSEN	348
2	human_E3 α I	HVEVLHSEIM	AHQKFALRLG	SWWNKI MSYS	SDFRQI FCQA	CLREEPDSEN	348
	Consensus	.V.V.HS...	AHQ.F.L.L.	SW...I...YS	...R.I.CQ.	.L.E.PD.EN	350

Figure 1C

6	mouse_E3 α II	SSLVDRLMLN	DSKLWKGARS	VYHQLFMSSL	LMDLKYKKLF	ALRFAKNYRQ	398
4	human_E3 α II	SSLVDRLMLS	DSKLWKGARS	VYHQLFMSSL	LMDLKYKKLF	AVRFAKNYQQ	398
15	mouse_E3 α I	PCLISRLMLW	DAKLYKGARK	ILHELIFSSF	FMEMEYKKLF	AMEFVKYYKQ	398
2	human_E3 α I	PCLISRLMLW	DAKLYKGARK	ILHELIFSSF	FMEMEYKKLF	AMEFVKYYKQ	398
	Consensus	..L..RLML.	D.KL.KGAR.	..H.L..SS.	.M..YKKLF	A..F.K.Y.Q	400
6	mouse_E3 α II	LQRDFMEDDH	ERAVSVTALS	VQFFTAPTAL	RMLLTEENLM	TVIIKAFMDH	448
4	human_E3 α II	LQRDFMEDDH	ERAVSVTALS	VQFFTAPTAL	RMLITEENLM	SIIIKTFMDH	448
15	mouse_E3 α I	LQKEYISDDH	ERSISITALS	VQMLTVPTLA	RHLIEEQNVI	SVITETLLEV	448
2	human_E3 α I	LQKEYISDDH	DRSISITALS	VQMFTVPTLA	RHLIEEQNVI	SVITETLLEV	448
	Consensus	LQ.....DDH	ER..S.TALS	VQ.FT.PTLA	R.LI.E.N..	SVI..T...	450

[illegible]

Figure 1E

6	mouse_E3 α II	I RYCVS	QEKV	SIHLPI	SRLL	AGLHV	LLSKS	EVAYKF	PELL	PLSEL	SPPM	648
4	human_E3 α II	I YCVS	QEKV	SIHLPV	SRLL	AGLHV	LLSKS	EVAYKF	PELL	PLSEL	SPPM	648
15	mouse_E3 α I	KSYKV	SEDLV	SIHLPL	SRTL	AGLHV	RLSRL	GAISRL	HEFV	PFDSF	QVEVL	645
2	human_E3 α I	KSYRV	SEDLV	SIHLPL	SRTL	AGLHV	RLSRL	GAISRL	HEFV	SFEDF	QVEVL	645
	Consensus	..Y.VS...	V	SIHLP.	SR.L	AGLHV.	LS..E..	P.....	L		650
6	mouse_E3 α II	I EHPL	RCLVL	CAQVH	AGMMR	RNGFS	LVNQI	YYYHN	VKCR	EMFDK	DI VML	698
4	human_E3 α II	I EHPL	RCLVL	CAQVH	AGMMR	RNGFS	LVNQI	YYYHN	VKCR	EMFDK	DVVM	698
15	mouse_E3 α I	VEYPL	RCLVL	VAQVVA	EMMR	RNGLS	LSI SQV	FYYQD	VKCR	EMYDK	DI I ML	695
2	human_E3 α I	VEYPL	RCLVL	VAQVVA	EMMR	RNGLS	LSI SQV	FYYQD	VKCR	EMYDK	DI I ML	695
	Consensus	.E.PLR	RCLVL	.AQV.A.	MMR	RNG.SL..	Q..YY..	VKCR.	EM	DKDI.	ML	700
6	mouse_E3 α II	QTGV	SMDPN	HFLM	MLSRF	ELYQL	FSTPD	YGKRF	SSEVT	HKDVV	QQNNT	748
4	human_E3 α II	QTGV	SMDPN	HFLM	MLSRF	ELYQI	FSTPD	YGKRF	SSEIT	HKDVV	QQNNT	748
15	mouse_E3 α I	QI GAS	MDPN	KFLLL	VLQRY	EL----	TDA	FNKTI	ST--K	DQDLI	KQYNT	738
2	human_E3 α I	QI GAS	MDPN	KFLLL	VLQRY	EL----	AEA	FNKTI	ST--K	DQDLI	KQYNT	738
	Consensus	Q.G.S.	MDPN	.FL...L.R.	EL.....T...	..K..S.....	..D...Q.	NT				750

Figure 1F

6	mouse_E3 α II	LI EEMLYLII	MLVGERFNP	VGQAATDEI	KREI I HQLSI	KPMAHSELVK	798
4	human_E3 α II	LI EEMLYLII	MLVGERFSP	VGQVNATDEI	KREI I HQLSI	KPMAHSELVK	798
15	mouse_E3 α I	LI EEMLQVLI	YI VGERYVPG	VGNVTREEVI	MREI THLLCI	EPMPHSAI AR	788
2	human_E3 α I	LI EEMLQVLI	YI VGERYVPG	VGNVTKEEVT	MREI I HLLCI	EPMPHSAI AK	788
	Consensus	LI EEM...I	.. VGER.. PG	VG. V....I	. REI I H. L. I	. PM HS... K	800
6	mouse_E3 α II	SLPEDENKET	GMESVI ESVA	HFKKPGLTGR	GMVELKPECA	KEFNLYFYHF	848
4	human_E3 α II	SLPEDENKET	GMESVI EAVA	HFKKPGLTGR	GMVELKPECA	KEFNLYFYHF	848
15	mouse_E3 α I	NLPENENNET	GLENVI NKVA	TFKKPGVSGH	GVYELKDESL	KDFNMVIFYHY	838
2	human_E3 α I	NLPENENNET	GLENVI NKVA	TFKKPGVSGH	GVYELKDESL	KDFNMVIFYHY	838
	Consensus	. LPE. EN. ET	G. E. VI .. VA	. FKKPG. . G.	G. YELK. E..	K. FN. YFYH.	850
6	mouse_E3 α II	SRAEQSKAEE	AQRKLKRENK	EDTALPPPAL	PPFCPLFASL	VNI LQCDVML	898
4	human_E3 α II	SRAEQSKAEE	AQRKLKQNR	EDTALPPPV	PPFCPLFASL	VNI LQSDVML	898
15	mouse_E3 α I	SKTQHSKAEH	MQKKRRKQEN	KDEALPPPPP	PEFCPAFSKV	VNLLSCDVM	888
2	human_E3 α I	SKTQHSKAEH	MQKKRRKQEN	KDEALPPPPP	PEFCPAFSKV	I NLLNCDI MM	888
	Consensus	S.... SKAE.	. Q. K... Q..	. D. ALPPP..	P. FCP. F...	VN. L. CDVM	900

Figure 1G

SEQ ID NO:					
6	mouse_E3 α II	YI MGTI LQWA	VEHHGSWSE	SMLQRVLHLI	GMALQEEKHH LENAEGHVQ 948
4	human_E3 α II	CI MGTI LQWA	VEHNGYAWSE	SMLQRVLHLI	GMALQEEKQH LENVTEEHV 948
15	mouse_E3 α I	YI LRTI FERA	VDTESNLWTE	GMLQMAFHI L	ALGLLEEKQQ LQKAPEEEV- 937
2	human_E3 α I	YI LRTV FERA	I DTDSNLWTE	GMLQMAFHI L	ALGLLEEKQQ LQKAPEEEV- 937
	Consensus	YI...TI...A	V.....WE	.MLQ...H... .	.L.EEKQ. L..A.EE.V. 950
6	mouse_E3 α II	TFTFTQKI SK	PGDAPHNSPS	ILAMLETQLN	APSLAETHKDM I RWLLKMFNA 998
4	human_E3 α II	TFTFTQKI SK	PGEAPKNSPS	ILAMLETQLN	APYLEVHHKDM I RWLKTFFNA 998
15	mouse_E3 α I	AFDFYHKASR	LGSSAMNAQN	I QMLLERLKG	IPQLEGQKDM ITWLQMFDT 987
2	human_E3 α I	TFDFYHKASR	LGSSAMNI QM	L---LEKLKG	IPQLEGQKDM ITWLQMFDT 984
	Consensus	TF.F..K.S.	.G....N... I...	LE.L... .P.LE..KDM I.WL.MF..	1000
6	mouse_E3 α II	IKKI RE--CS	SSSPVAEAE	TI MEESSRDK	DKAERKRKAE I ARLRREKI M 1046
4	human_E3 α I	VKKMRE--SS	PTSPVAETEG	TI MEESSRDK	DKAERKRKAE I ARLRREKI M 1046
15	mouse_E3 α I	VKRLREKSCL	VVATTSGLEC	IKSEETHDK	EKAERKRKAE AARLHRQKI M 1037
2	human_E3 α I	VKRLREKSCL	I VATTSGSES	IKNDEITHDK	EKAERKRKAE AARLHRQKI M 1034
	Consensus	VK..RE..C.E.	...EE...DK	.KAERKRKAE .ARL.R.KI M 1050

Figure 1H

6	mouse_E3 α II	AQMSEMQRHF	I	DENKELFQQ	TLELDTSASA	TL--	DSSPPV	SDAALTALGP	1094
4	human_E3 α II	AQMSEMQRHF	I	DENKELFQQ	TLELDASTA	VL--	DHSPVA	SDMTLTALGP	1094
15	mouse_E3 α I	AQMSALQKNF	I	ETHKLMYDN	TSEVTGKEDS	I	MEESTSAV	SEASRIALGP	1087
2	human_E3 α I	AQMSALQKNF	I	ETHKLMYDN	TSEMPGKEDS	I	MEESTPAV	SDYSRIALGP	1084
	Consensus	AQMS..Q..F	I...	K.....	T.E.....	S.P.V	SD....ALGP	1100
6	mouse_E3 α II	AQTQVPEPRQ	F	VTCLLCQEE	QEVTVGSRAM	VLA	AFVQRST	VLSKDRTKTI	1144
4	human_E3 α II	TQTQVPEQRQ	F	VTCLLCQEE	QEVKVESRAM	VLA	AFVQRST	VLSKNRSKFI	1144
15	mouse_E3 α I	KRGPAVTEKE	V	LTCILCQEE	QEVKLENNAM	VLS	ACVQKST	ALTQHRGKPV	1137
2	human_E3 α I	KRGPSVTEKE	V	LTCILCQEE	QEVKIENNAM	VLS	ACVQKST	ALTQHRGKPI	1134
	Consensus	TCILCQEE	QEVK.E..	AM	VL.A.VQ.ST	.L...R.K.I	1150
6	mouse_E3 α II	AD-PEKYDPL	F	MHPDLSCGT	HTGSCGHVMH	AHC	WQRYFDS	VQAKEQRRQQ	1193
4	human_E3 α II	QD-PEKYDPL	F	MHPDLSCGT	HTSSCGHI	MH	AHCWQRYFDS	VQAKEQRRQQ	1193
15	mouse_E3 α I	DHLGETLDPL	F	MDPDLAHGT	YTGSCGHVMH	AVC	WQKYFEA	VQ---LSSQQ	1184
2	human_E3 α I	ELSGEALDPL	F	MDPDLAYGT	YTGSCGHVMH	AVC	WQKYFEA	VQ---LSSQQ	1181
	ConsensusE..DPL	FM	PDL..GT	.TGSCGHVMH	A.CWQ	YF..	VQ.....QQ	1200

Figure 1I

6	mouse_E3 α II	RLRLHTSYDV	ENGEFLCPLC	ECLCNTVIPL	L-LPPRSILS	RRLN-FSDQP	1241
4	human_E3 α II	RLRLHTSYDV	ENGEFLCPLC	ECLCNTVIPL	L-LPPRNIFN	NRLN-FSDQP	1241
15	mouse_E3 α I	RIHVDL-FDL	ESGEYLCPLC	KSLCNTVIPI	IPLQPQKINS	ENAEALAQLL	1233
2	human_E3 α I	RIHVDL-FDL	ESGEYLCPLC	KSLCNTVIPI	IPLQPQKINS	ENADALAQLL	1230
	Consensus	R.....D	E.GE.LCPLC	..L.NTVIP.	..L.P..I.S	1250
6	mouse_E3 α II	DLAQWTRAVT	QQIKVVQMLR	RKHNAA-DTS	SSEDTEAMNI	IPIPEGFRPD	1290
4	human_E3 α II	NLTQWRTIS	QQIKALQFLR	KEESTP-NNA	STKNSENVDE	LQLPEGFRPD	1290
15	mouse_E3 α I	TLARWQTVL	ARISGYNIKH	AKGEAPAVPV	LFNQGMGDST	FEFHSILSFG	1283
2	human_E3 α I	TLARWQTVL	ARISGYNI RH	AKGENP-IPI	FFNQGMGDST	LEFHSILSFG	1279
	Consensus	.LA.W.TV.	..I.....	.K...P-...	1300
6	mouse_E3 α II	FYPRNPYSDS	I KEMLTTFGT	AAYKVGLKVH	PNEGDPVRPI	LCWGTCAVTI	1340
4	human_E3 α II	FRPKI PYSES	I KEMLTTFGT	ATYKVGLKVH	PNEEDPRVPI	MCWGSCAYTI	1340
15	mouse_E3 α I	VQSSVKYSNS	I KEMVILFAT	TIYRI GLKVP	PDELDPRVPM	MTWSTCAFTI	1333
2	human_E3 α I	VESSI KYSNS	I KEMVILFAT	TIYRI GLKVP	PDERDPRVPM	LTWSTCAFTI	1329
	ConsensusYS.S	I KEM...F.T	..Y..GLKV.	P.E.DPRVP.	..W.TCA.TI	1350

[illegible]

Figure 1K

6	mouse_E3 α II	LHKTLLHQYTG	SALKEAPSGW	HLWRSVRAAI	MPFLKCSAL	FHYLNGVPAP	1532
4	human_E3 α II	LYKTLHQYTG	SALKEIPSGW	HLWRSVRAGI	MPFLKCSALF	FHYLNGVPSP	1532
15	mouse_E3 α I	FFVEVSQHTD	GLTGCGAPGW	YLWLSLRNGI	TPYLRCAALL	FHYLLGVAPP	1533
2	human_E3 α I	FFAEISQYTS	GSIGCDIPGW	YLWVSLKNGI	TPYLRCAALF	FHYLLGVTPP	1525
	ConsensusQYT.GW	.LW.S.R.GI	.P.L.C.AL	FHYL.GV..P	1550
6	mouse_E3 α II	PDLQV-SGTS	HFEHLCNYLS	LPTNLIHLFQ	ENSDIMNSLI	ESWCQNSEVK	1581
4	human_E3 α II	PDIQV-PGTS	HFEHLCSYLS	LPNNLICLFQ	ENSEIMNSLI	ESWCNNEVK	1581
15	mouse_E3 α I	EELFANSAEG	EFSALCSYLS	LPTNLFLLFQ	EYWDTIRPLL	QRWCGDPALL	1583
2	human_E3 α I	EELHTNSAEG	EYSALCSYLS	LPTNLFLLFQ	EYWDTVRPLL	QRWCADPALL	1575
	Consensus	..L...S...	.F..LCSYLS	LPTNL..LFQ	E...D....L.	..WC.....	1600
6	mouse_E3 α II	RYLNGERGAI	SYPRGANKLI	DLPEDYSSLI	NQASNFSCPK	SGGDKSRAPT	1631
4	human_E3 α II	RYLEGERDAI	RYPRESNKLI	NLPEDYSSLI	NQASNFSCPK	SGGDKSRAPT	1631
15	mouse_E3 α I	KSLKQKSAVV	RYPRKRNSLI	ELPEDYSCLL	NQASHFRCPR	SADDERKHPV	1633
2	human_E3 α I	NCLKQKNTVW	RYPRKRNSLI	ELPDDYSCLL	NQASHFRCPR	SADDERKHPV	1625
	Consensus	..L.....	RYPR...N.LI	.LPEDYS.L.	NQAS.F.CP.	S...D....P.	1650

Figure 1L

6	mouse_E3 α II	LCLVCGSLLC	SQSYCCQAEI	EGEDVGACTA	HTYSCGSGAG	I FLRVRECQV	1681
4	human_E3 α II	LCLVCGSLLC	SQSYCCQTEL	EGEDVGACTA	HTYSCGSGVG	I FLRVRECQV	1681
15	mouse_E3 α I	LCLFCGAILC	SQNI CCQEIV	NGEVVGACVF	HALHCGAGVC	I FLKI RECRV	1683
2	human_E3 α I	LCLFCGAILC	SQNI CCQEIV	NGEVVGACIF	HALHCGAGVC	I FLKI RECRV	1675
	Consensus	LCL. CG. . LC	SQ. . CCQ. . .	. GE. VGAC. .	H. . . CG. GV.	I FL. . REC. V	1700
6	mouse_E3 α II	LFLAGKTKGC	FYSPPYLDDY	GETDQGLRRG	NPLHLCQERF	RKI QKLWQQH	1731
4	human_E3 α II	LFLAGKTKGC	FYSPPYLDDY	GETDQGLRRG	NPLHLCKERF	KKI QKLWHQH	1731
15	mouse_E3 α I	VLVEGKARGC	AYPAPYLDEY	GETDPGLKRG	NPLHLSRERY	RKLHLVWQQH	1733
2	human_E3 α I	VLVEGKARGC	AYPAPYLDEY	GETDPGLKRG	NPLHLSRERY	RKLHLVWQQH	1725
	Consensus GK. . . GC	. Y. . PYLD. Y	GETD. GL. RG	NPLHL. . ER.	RK. . . . WQQH	1750
6	mouse_E3 α II	SITEEI GHAAQ	EANQTLVGI D	WQHL			1755
4	human_E3 α II	SVTEEI GHAAQ	EANQTLVGI D	WQHL			1755
15	mouse_E3 α I	CIIEEI ARSQ	ETNQMLFGFN	WQLL			1757
2	human_E3 α I	CIIEEI ARSQ	ETNQMLFGFN	WQLL			1749
	Consensus	. I. EEI . . . Q	E. NQ. L. G. .	WQ. L			1774

Tth Expression Profile of huE3 α -II in Human Tissues

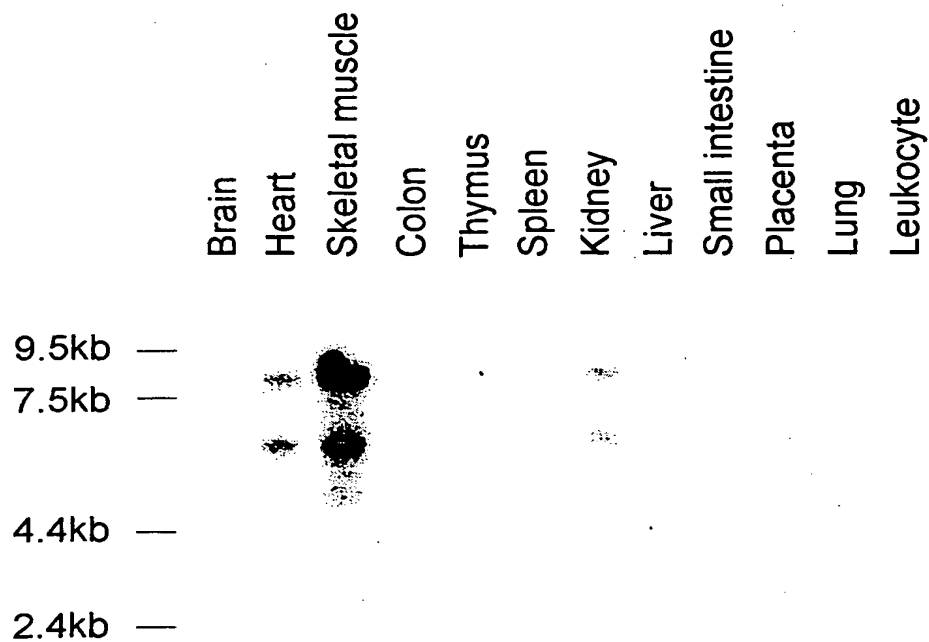


FIG. 3

Tth Expression Profile of huE3 α -I in Human Tissues

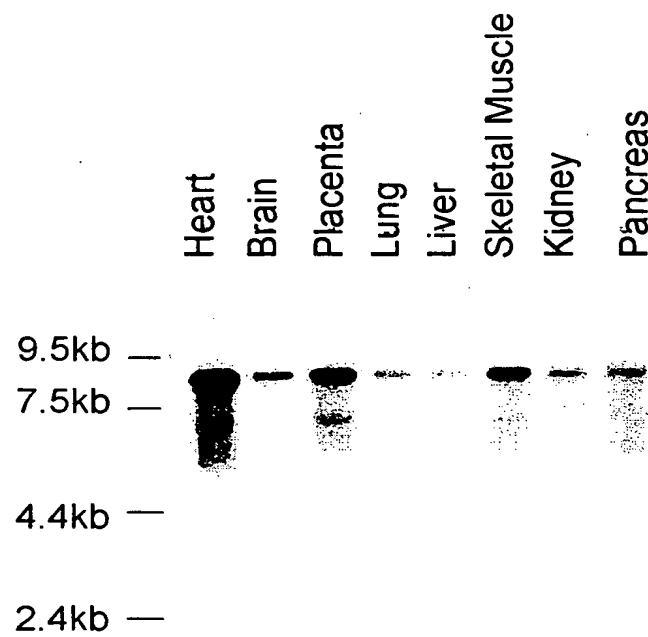


Figure 4
Ubiquitination of Endogenous Proteins

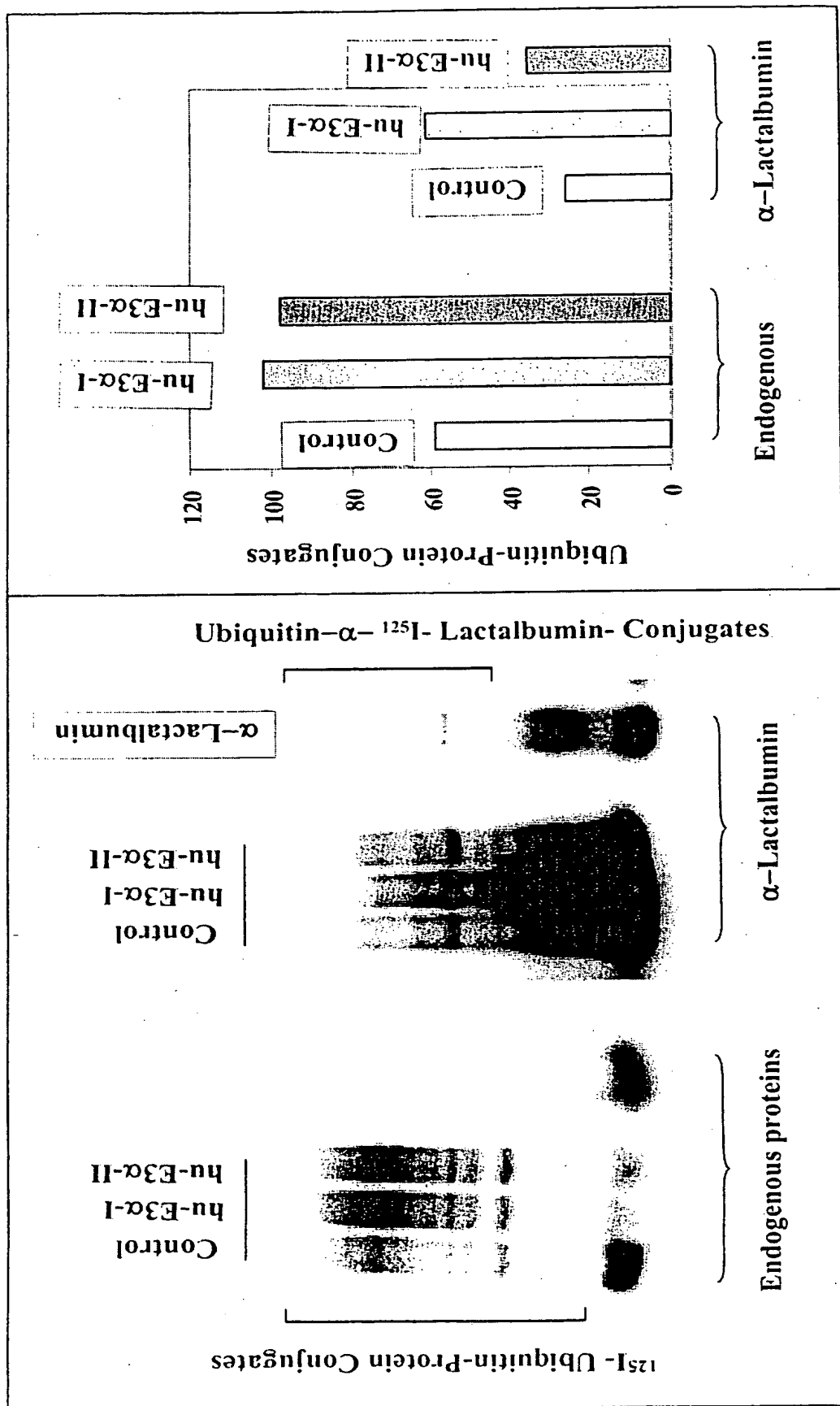


Figure 5
Transfection of Human E3 α -I or E3 α -II cDNA Stimulates
Ubiquitin Conjugation in Cultured Muscle Cell Lines

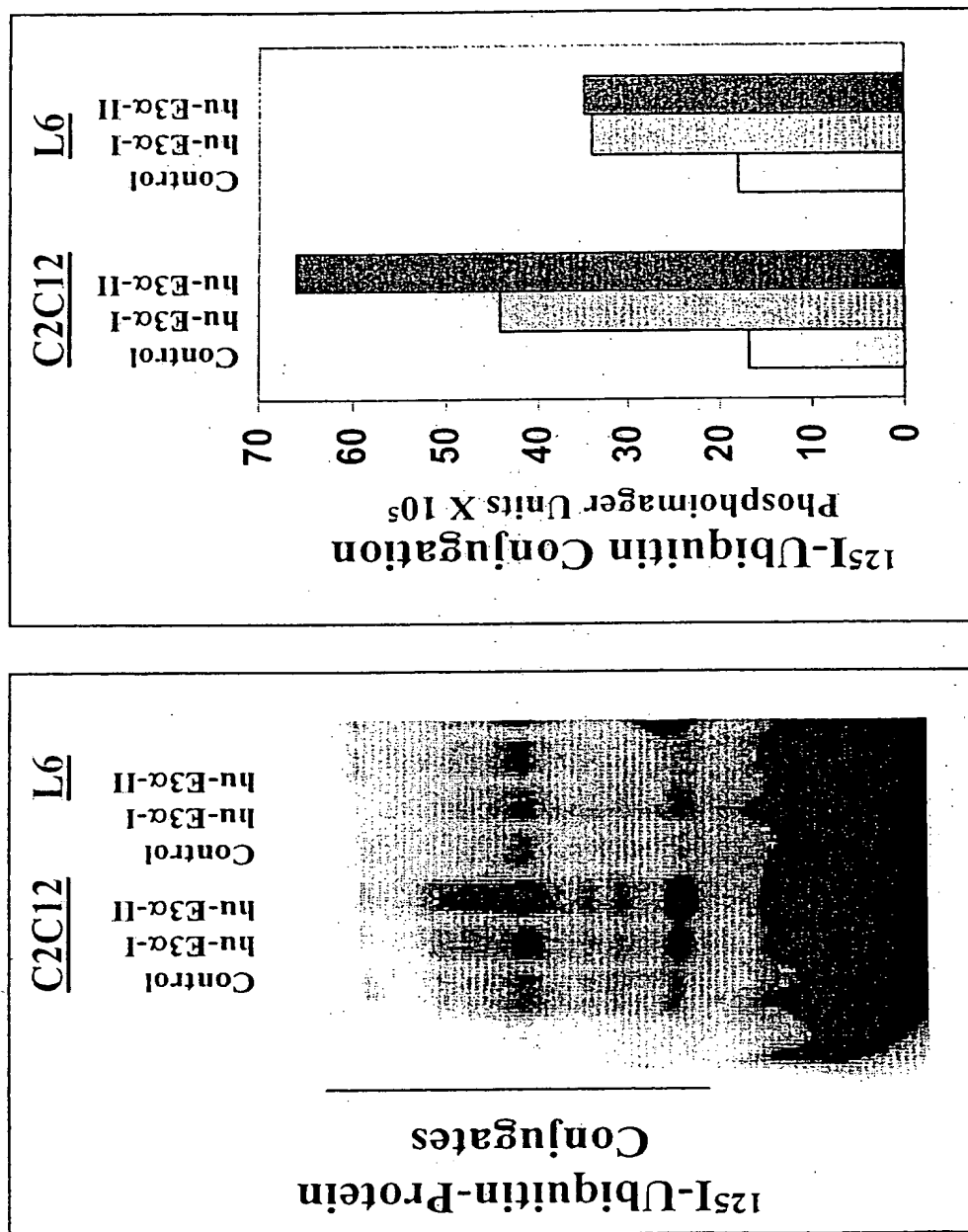


Figure 6
 125 I-Ubiquitin Conjugation to Muscle Proteins and Its Sensitivity to E3 α Inhibitor
in Skeletal Muscle Extracts

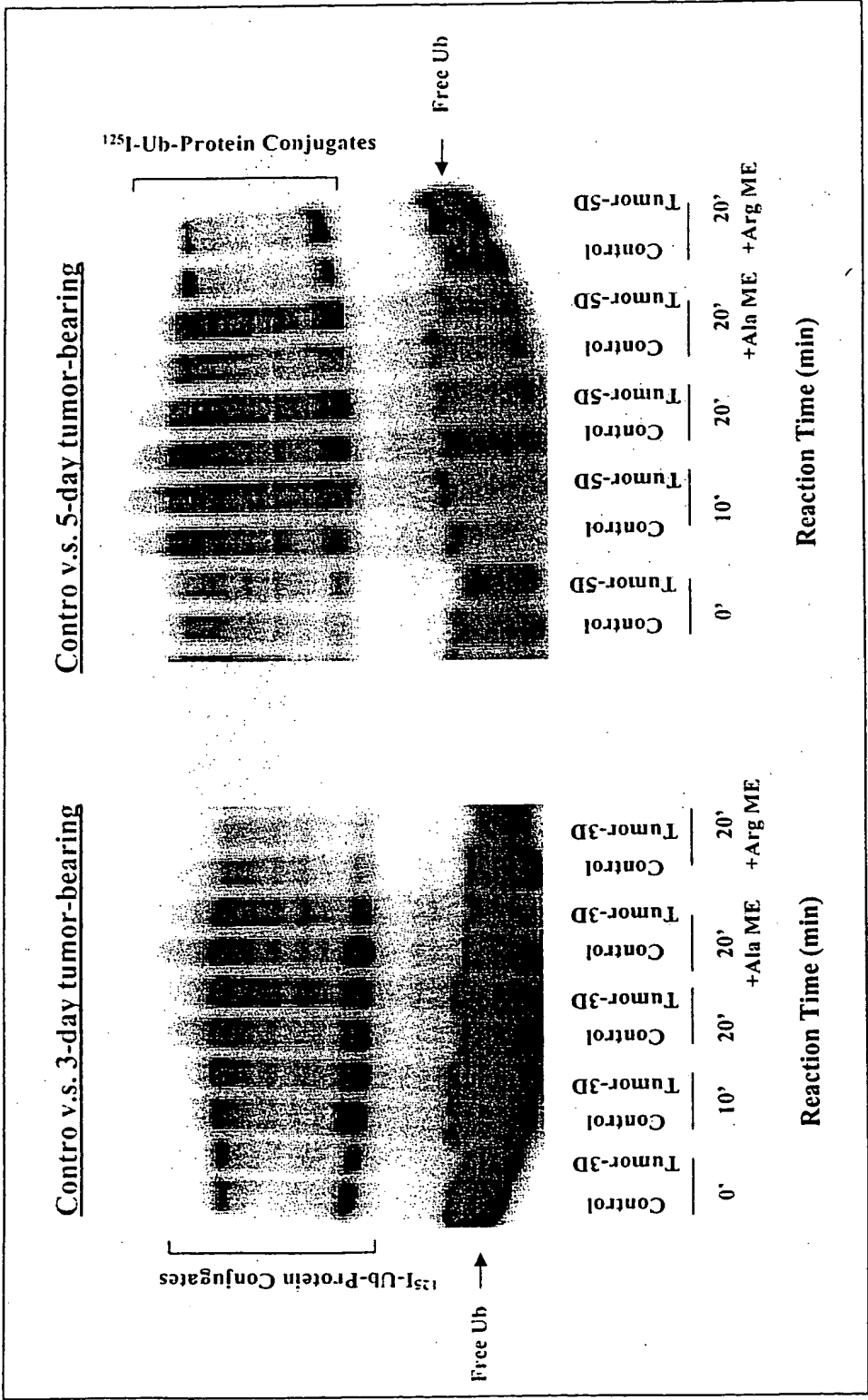


Figure 7
 Rates of Ubiquitination of N-end Rule Substrate
 α -Lactalbumin in Skeletal Muscle Extracts

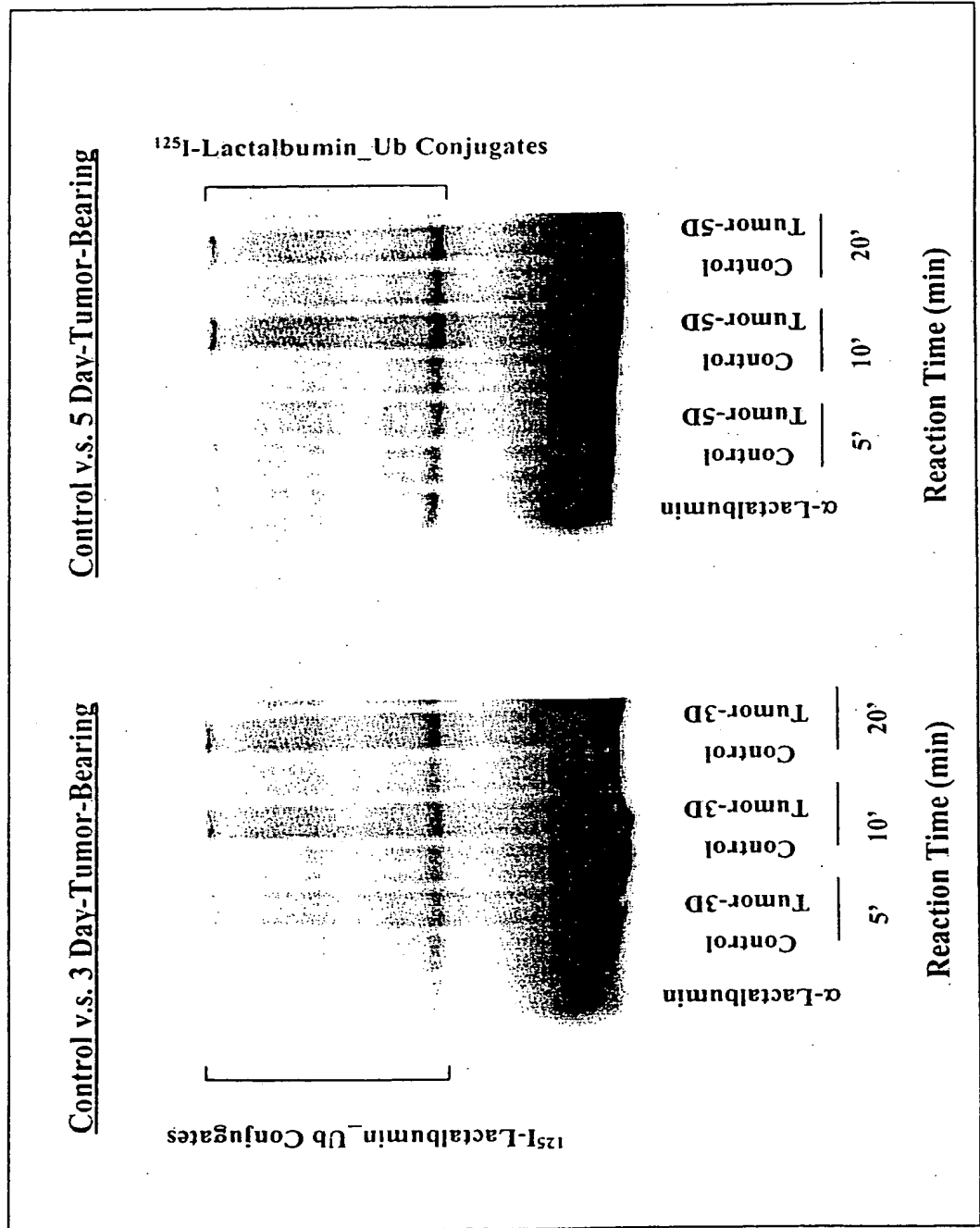


Figure 8
Northern blot analysis of E3 α -I & E3 α -II expression
in gastrocnemius muscles in YAH-130 experimental cachexia model

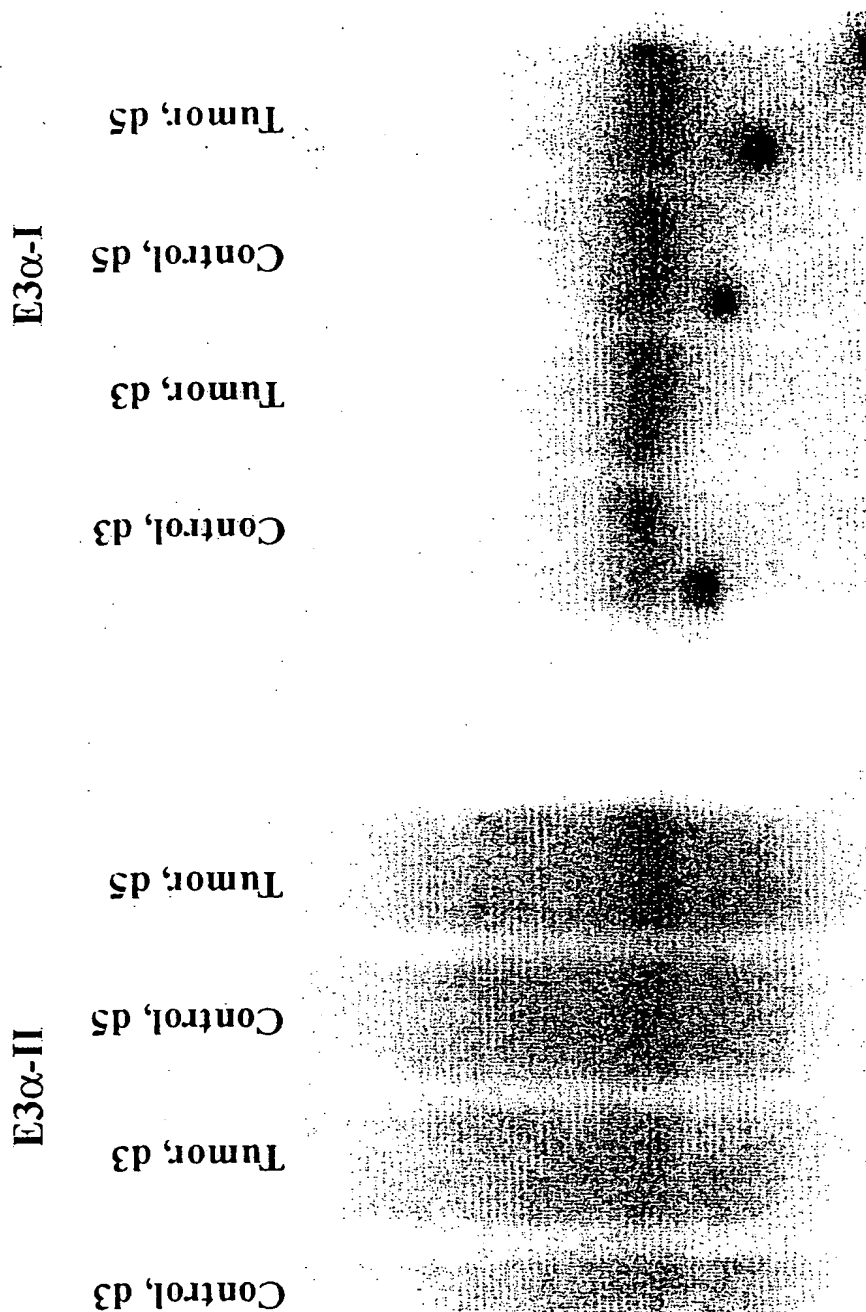


Figure 9

**Northern blot analysis of E3 α -I and E3 α -II expression in
 gastrocnemius muscle and cardiac muscle
 in C26 experimental cachexia model**

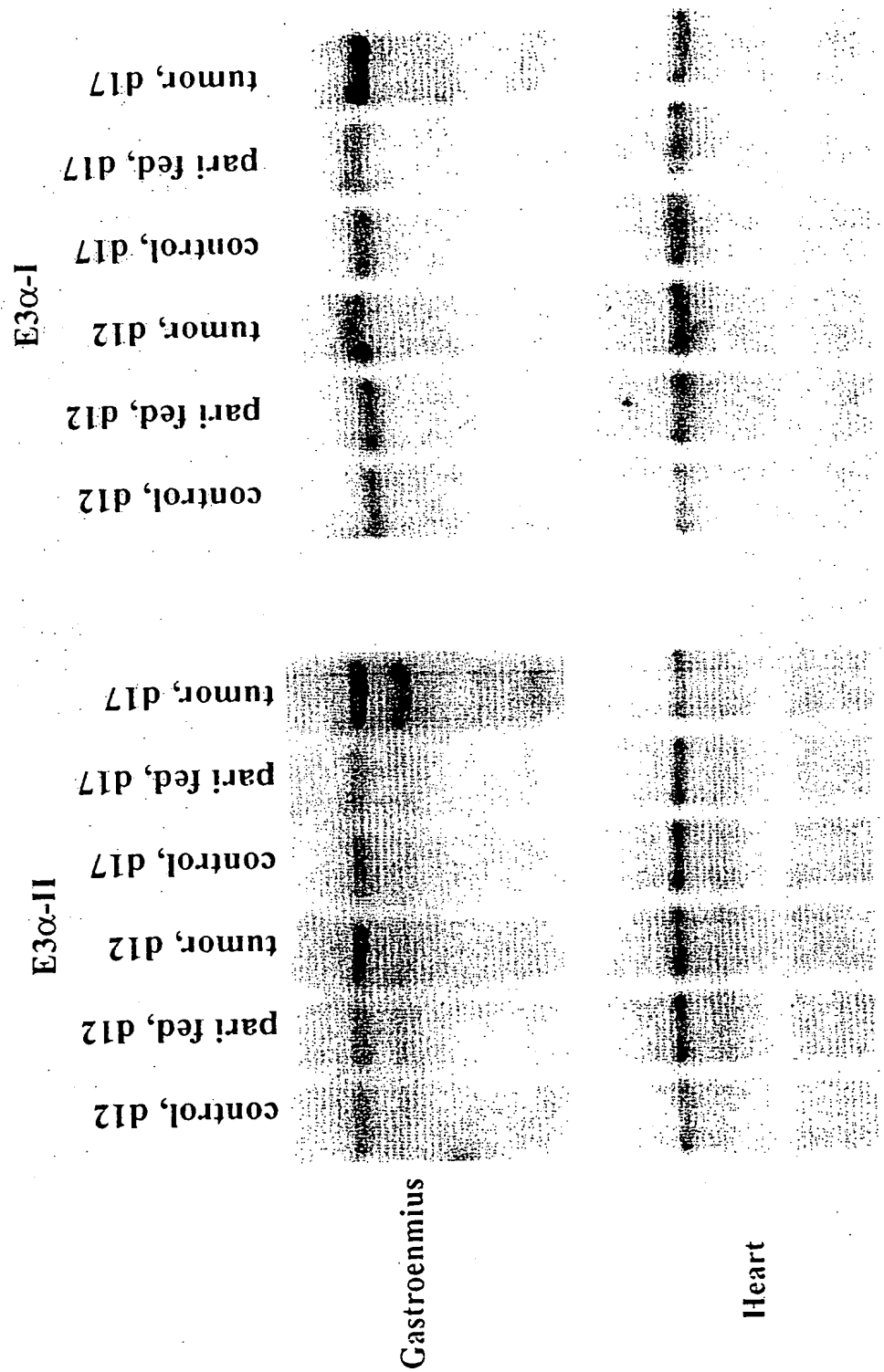


Figure 10
 Proinflammatory cytokines TNF- α and IL-6
 induce E3 α -II Expression in C2C12 myotube culture

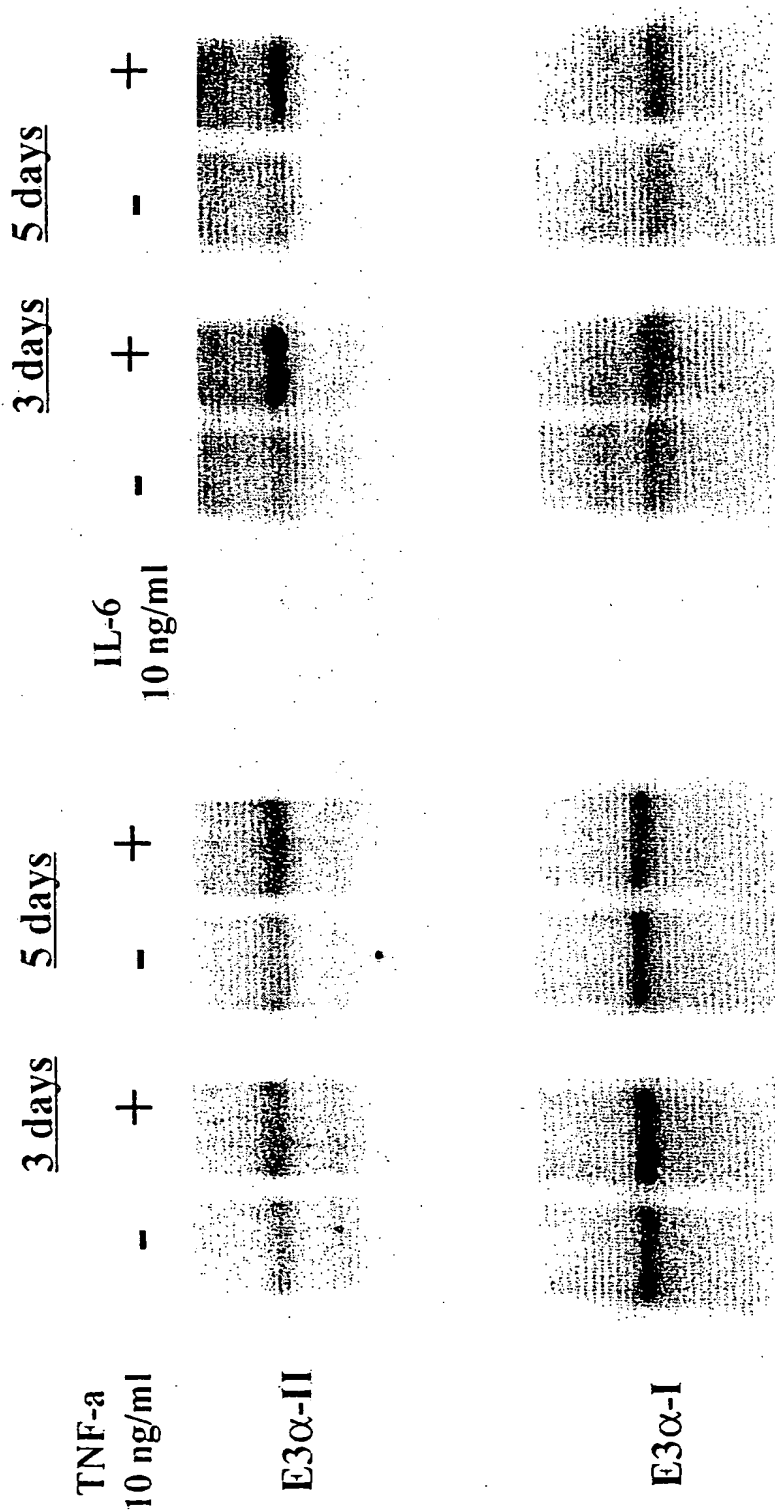


Figure 11
IL-6 Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures

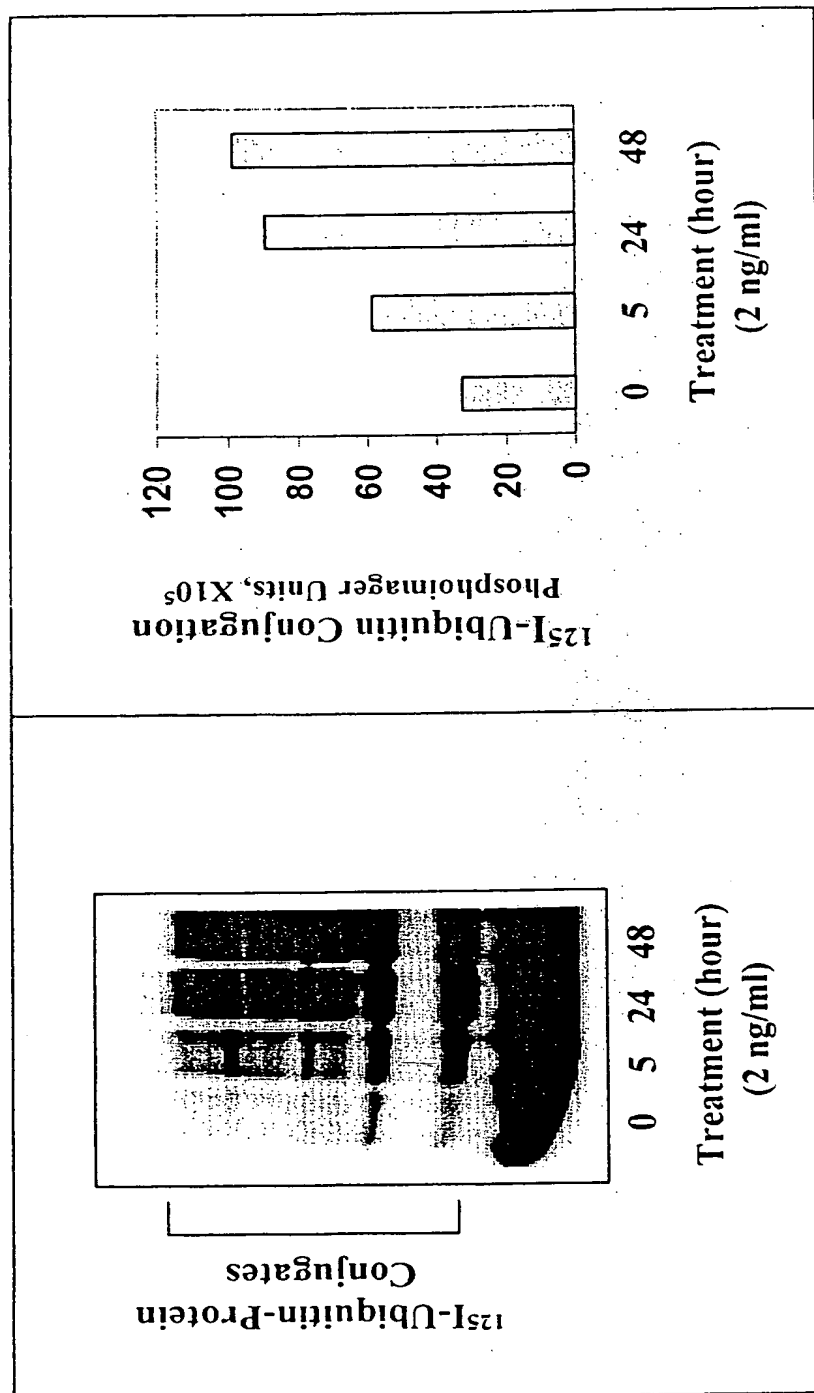


Figure 12
TNF α Elicits Accelerated Ubiquitination in C2C12 Myotube Cultures

